III. ANTIGEN/ANTIBODY TESTING FOR MALARIA INFORMATIONAL

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Malaria Antigen Detection Assays
FDA-CDRH Perspective

Regulatory Background:

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At CDRH, devices intended for *in vitro* diagnostic use are regulated under the authority of the Food, Drug, and Cosmetic Act, amended in May 1976 to add medical devices, in January 1990 to add the Safe Medical Devices Act, and 1997 to add the FDA Modernization Act.

In vitro diagnostic devices are classified under the act in Section 513 (a) (1). Class I and Class II devices usually can be compared or found "substantially equivalent" to another legally marketed device.

{Slide} Device Classes

CLASS I:
- General Controls –provide reasonable assurance of the safety and effectiveness of the device.
- The diagnostic use is not represented as being used to support life, or as having substantial importance in preventing impairment of human health.
- The diagnostic use does not present a potential unreasonable risk of illness or injury.

CLASS II:
- Special Controls – provide reasonable assurance of the safety and effectiveness of the device.
- General Controls are insufficient by themselves.
- Sufficient information available to develop performance criteria.

CLASS III:
- Insufficient information available to develop performance criteria.
- Usually no legally marketed predicate device.
- The diagnostic use is represented as being used to support life or as having substantial importance in the diagnosis of disease.
- The diagnostic use presents a potential risk of illness or injury.

There is usually sufficient information known about these devices, and controls, or guidance document exist that can be used to determine the performance of the device. For these type devices, manufacturers submit a premarket notification as described in Section (510k) of the Act. Class III devices however, are devices for which sufficient information is not available, and the device is of substantial importance in diagnosing or preventing serious illness. A premarket approval application (PMA) is submitted for Class III Devices.

Because sufficient information is not available on the performance of Malaria antigen detection, and they are of substantial importance in diagnosing and preventing life-threatening illness, CDRH has determined that these devices will be classified as Class III and a PMA will be required.

Microscopic examination of thin and thick blood films has been considered the standard reference method for diagnosing infection with *Plasmodium falciparum*. {Slide} In 1989, FDA/CDRH approved the QBC Malaria system as a qualitative screening method for detecting malaria parasites. This device was classified as a Class III Automated Differential Cell Counter. The device consisted of a QBC Tube that contained acridine orange stain and an anticoagulant. The QBC Tube was originally used for the quantitative determination of Hematocrit, Hemoglobin, WBC, Granulocyte, Lymphocyte. Monocyte, and Platelet Counts. The tubes are examined under a fluorescence microscope for detection of the acridine malaria organisms. This system does not differentiate or identify Plasmodium species.

FDA is aware that Malaria Antigen detection assays have been developed that capture *P. falciparum* antigen from a blood sample. There are a number of assays described in the literature that detect P. falciparum, and some P. vivax. The literature contains reference to monoclonal and polyclonal antibodies raised against specific excretory/secretory antigens (a heat-stable antigen Pf9 and a histidine-rich protein PfHRP-2) of *P. falciparum*, DNA probes for *P. falciparum*, and ELISA, IFA and IHA tests. However, none of these assays have been approved by the FDA.

A PMA for a Malaria Antigen detection test must therefore contain sufficient information to demonstrate that the device is safe and effective. The following review criteria have been developed by the FDA to guide manufacturers in conducting clinical studies that would yield sound scientific information that is clinically meaningful.

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REVIEW CRITERIA

I. Clinical Studies must be conducted to demonstrate that these assays are safe and effective for diagnostic use. The studies should support the Indications for Use of the Assay. Clinical Studies if not conducted in the U.S, must be conducted following the study protocols and the Helsinki Agreement. The following type studies

A. Validation of Cutoff values:

The clinical studies should challenge or validate the cutoff values determined in the pivotal studies.

B. Clinical Sensitivity

To determine the sensitivity of the assay for detecting P. falciparum or for differentiating P. vivax or other Plasmodium spp. the following type information should be provided:

1. Clearly defined populations (indigenous or endemic areas, low prevalent, etc) to reflect the intended use.
2. A clear description of how disease status was determined (e.g. clinical presentations, microscopic examination of thin/thick blood films) . Patient histories, to include symptoms, diagnosis, and other laboratory diagnosis are essential. Consent forms must be included.
3. A Protocol, which clearly defines the objectives of the study, exclusion/inclusion criteria, and the study design. All test methodologies, microscopic procedures, quality control and quality assurance methods must be developed and included.
4. The device should be tested at a minimum of three distinct geographical locations. Sites and investigators should be identified.

C. Clinical Specificity

To determine the specificity of the assay for detecting P. falciparum or for differentiating P. vivax or other Plasmodium spp. the following type information should be provided:

1. The population tested: which should include patients with microscopic evidence of other *Plasmodium* spp., other parasitemias, and other conditions with similar symptoms. A description of the methods used to determine disease conditions should be included.
2. Non-diseased patients may be included to challenge the specificity of the device.

(N.B. FDA will meet with manufacturers and review their clinical protocols before the studies are implemented.)

Non-Clinical Studies:

These studies are usually laboratory studies conducted to validate the assay and develop analytical information. The following information is required.

1. Characterization of components/Description of the Antigen, Antibodies. Description of Controls, Standards, or Calibrators,
2. Limits of Detection of the assay.
3. Determination of Cutoff Values for the assay

4. Reproducibility/Precision of the assay: This should include intra- and inter-assay, and lot-to-lot reproducibility. If the device is intended for Point-of-Care, reproducibility studies are conducted at representative sites.

Cross-Reactivity: For Malaria Antigen tests, this should include specimens from patients infected with other *Plasmodium* species, and other parasitemias of similar characteristics, and patients infected with microorganisms that effect similar symptoms, such as *Mycobacterium tuberculosis.*

Interference: from endogenous (hemoglobin, lipids, etc.) or exogenous substances (anticoagulants, temperature, etc.).

Stability data should stress the storage and shipping conditions.

Statistical Analysis when possible should include an analysis of Receiver Operating Curves (ROC) and consideration for use of equivocal zones to help minimize false positive and false negative results. Our Division looks forward to working with sponsors to help get new malarial diagnostics into the market.

Freddie M. Poole

"A rapid dipstick antigen capture assay for the diagnosis of falciparum malaria* - WHO Informal consultation on Recent Advances in Diagnostic Techniques and Vaccines for Malaria," ***Bulletin of the World Health Organization, 1996, 74 (1): 47-54.***

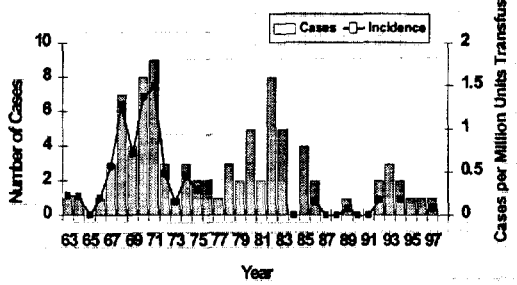
Preventing Transfusion-Transmitted Malaria: Role of Antigen/Antibody Testing

Division of Parasitic Diseases
Centers for Disease Control and Prevention

Preventing TTM: Current Guidelines

- Deferral for 3 years:
  - After diagnosis of malaria
  - Immigrants, refugees, residents arriving from endemic areas
  - *(Proposed: immigrants, refugees, prior residents of endemic areas returning from a visit to endemic areas)*
  - (Asymptomatic during interval)
- Deferral for 1 year:
  - Residents of non-endemic countries, returning from travel to endemic areas
  - (Asymptomatic during interval)

Incidence of Transfusion-Transmitted Malaria, 1963-1997



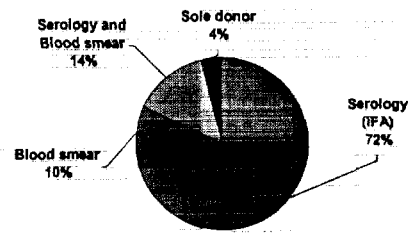
TTM in US

- 0.25 cases per million units collected
- 1963-1998:
  - 91 cases
  - 10 deaths
  - All 4 *Plasmodium* species
    - 35% *P. falciparum*
    - 29% *P. vivax*
    - 28% *P. malariae*
    - 4% *P. ovale*
    - 4% mixed or undetermined species

TTM, 1963-1998

- In 58 implicated donors with complete epidemiologic investigation:
  - 62% should have been excluded if the donor deferral guidelines had been correctly applied
  - 38% would not have been excluded; of these, 2/3 had *Plasmodium malariae*

TTM, 1963-1998
How 65 implicated donors were identified

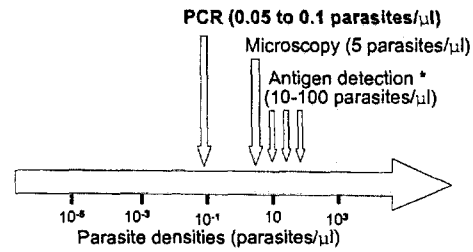


Note: serology and blood smears were most often performed using blood collected from the donors during the investigations

TTM, 1963-1998

- In implicated donors, where information available:
  - 33% had positive blood smear
  - 98% had positive serology

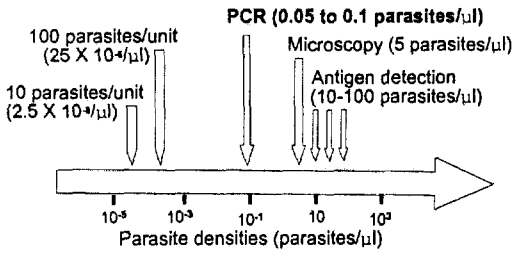
Preventing TTM: Detection of Parasites/Parasite Products



*: Some antigen detection tests do not detect all four species of human *Plasmodium*

CDC

Preventing TTM: Detection of Parasites/Parasite Products



Detection of 10 parasites/unit requires a sensitivity:
-4,000 times better than PCR
-200,000 times better than microscopy

CDC

Preventing TTM: Antibody Detection

- Advantages:
  - In investigations of implicated donors, serology has proven most effective
  - Infection is followed by seroconversion in 1-2 weeks, usually during, or shortly following residence/travel in endemic areas

Preventing TTM: antibody detection

- Disadvantages:
  - Positivity can persist for years following cure (seropositivity rate unknown among returning travelers/immigrants)
- Technical issues of mass screening:
  - Antigen (not crude parasite extracts; reactivity for 4 species)
  - Automation (ELISA, not IFA)
  - Which population to screen?

Preventing TTM: France

- History of malaria: defer indefinitely
- Returning from endemic area:
  - defer for 4 months following return
  - between 4 months and 3 years following return: AB testing
- AB testing:
  - IFA ("in house" or commercial kit)
  - ELISA earlier (unsatisfactory results with commercial kit)

Preventing TTM: United Kingdom
(April 1998-present)

- Visitors returning from endemic areas:
  - defer for 6 months following return
  - 6-12 months: AB testing
  - after 12 months: no AB testing needed
- "Residents"* arriving from endemic areas:
  - defer for 6 months following arrival
  - after 6 months: AB testing
  - if AB testing unavailable: defer

*: *"Resident" = lived first 5 years in endemic area for at least 3 months*

Preventing TTM: United Kingdom
(April 1998-present)

- History of malaria (proven or suspected):
  - defer for 6 months following episode
  - after 6 months: AB testing
- AB testing:
  - mostly by ELISA, using commercially available kit (except Scotland)
  - if positive AB test: defer indefinitely (or until AB test reverts to negative)
  - if no AB testing available: consider as positive

Preventing TTM: Summary

- Current US criteria (history-based) do not always prevent TTM
- Current testing methods for parasites or parasite products (including antigens) are not sensitive enough for detecting theoretical infective doses
- However: no data on actual parasite densities in incriminated blood units

Preventing TTM: Summary

- During investigations of TTM, antibody testing has been more effective for detecting infected donors
- But AB testing would result in deferral of donors whose serology is positive due to past (and cured) malaria
- However: no data on serological positivity rate in US blood donors

Preventing TTM: Summary

- In France and the United Kingdom (except Scotland), serology is used as a screening tool in selected donor groups
- If mass screening using serology were adopted, it would require an automated system (ELISA) using practical antigens (recombinant proteins or peptides)